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GUAIANOLIDES FROM *SAUSSUREA AFFINIS*

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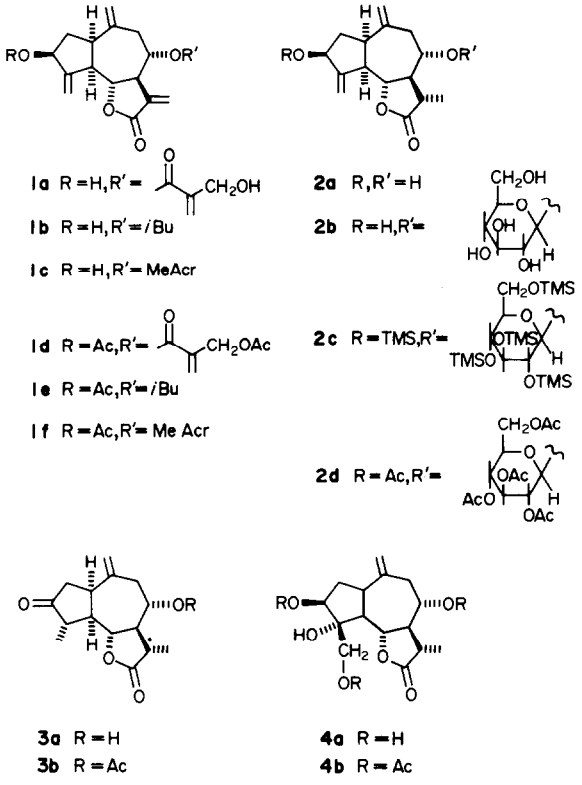
Key Word Index—*Saussurea affinis*; Cardueae; Compositae; sesquiterpene lactones; guaianolides.

Abstract—Cynaropicrin, 11βH-11,13-dihydrodesacylcynaropicrin, aguerins A and B, isoamberboin and the new guaianolides saussureolide and 11βH-11,13-dihydrodesacylcynaropicrin 8-β-D-glucoside were isolated from *Saussurea affinis*.

INTRODUCTION

Commercially available costus root oil from *Saussurea lappa* yields the germacradienolide costunolide and transformation products belonging to the elemadienolide, eudesmanolide and guaianolide series [1–3] whereas the above-ground parts of a few other representatives of the large genus *Saussurea* have furnished primarily a group of guaianolides related to cynaropicrin (**1a**) [1,4–9]. We now report the results of our examination of *Saussurea affinis* whose range extends from north-east India to the Far East. The compounds isolated were cynaropicrin (**1a**), which is widely distributed in Cardueae [16], diol **2a** previously isolated from *Tricholepis glaberrima* [10]‡, aguerin A (**1b**) and aguerin B (**1c**) which are found in several *Centaurea* species [11–14] and in *Cousinia onopordioides* [15], isoamberboin (**3a**) previously isolated from *Jurinea maxima* [17], and the new guaianolides **2b** and **4a**.



‡The C-11 epimer of **2a** has recently been isolated from *Ainsliaea fragrans* [18].

RESULTS AND DISCUSSION

One very polar new lactone was a β-D-glucoside ($J_{1',2'}$ = 7.5 Hz) for which structure **2b** could be postulated on the basis of the ^{1}H NMR spectra of its TMS ether **2c** and its penta-acetate, **2d** (Table 1). The entire sequence of protons attached to the guaianolide carbon skeleton was established by extensive spin decoupling. Enzymatic hydrolysis furnished **2a** [10] and glucose. The glucose moiety was attached to the oxygen function on C-8 and not to that on C-3 because of the pronounced chemical shift of the H-3 signal to lower field on conversion of **2b** to **2d**.

A second new somewhat less polar substance was the tetrol, **4a**, which we have named saussureolide. Comparison of the ^{1}H NMR spectrum of **4a** with that of its triacetate **4b** and extensive decoupling studies on the latter showed that the carbon skeleton and stereochemistry at the relevant centers were identical with those of its congeners **1a**–**1c** and **2a**. However, in the new compound the exocyclic methylene on C-4 was replaced by $-CH_2OH$ which, because of the multiplicities of the signals involved, had to be attached to a quaternary carbon. The presence of a vicinal tertiary hydroxyl on C-4 was inferred from the mass spectrum and the IR spectrum of **4b** which continued to display hydroxyl absorption, and was confirmed by the ^{13}C NMR spectrum (Table 2). The stereochemistry at C-4 was deduced by examination of the ^{1}H NMR spectrum of **4b** after reaction with trichloroacetylisocyanide (TAI) [19]; in the spectrum of the resulting monocarbamate (Table 1) the signals of H-3 and H-5 had moved to considerably lower field. Hence, the tertiary hydroxyl was *cis* to H-3 and H-5 and α.

Table 1. ^{1}H NMR spectra* of compounds **2c**, **2d**, **4a** and **4b**

H No.	2c	2d	4a§	4b	4b + TAI
1	2.82 *br q* (9)	2.98 *br q*	3.06 *br q*	3.21 *br q*	3.30 *br q*
2a	2.08 *ddd* (12, 8, 7)	2.43 *m*	2.19 *ddd* (14, 10, 7.5)	2.32 *ddd*	2.47 *m*
2b	1.66 *ddd* (12, 8, 7)	1.77 *ddd*	1.81 *ddd* (14, 10, 7.5)	1.78 *ddd*	1.91 *ddd*
3	4.43 *br t* (7)	5.53 *br t*	4.10 *t* (7.5)	5.07 *t*	5.81 *t*
5	2.73 *br t* (7)	2.80 *br t*	2.26 *t* (10.5)	2.43 *t*	3.44 *t*
6	4.01 *t* (10)	3.98 *dd* (10, 9)	4.21 *dd* (10.5, 9.5)	4.23 *dd*	4.33 *dd*
7	2.20 *q* (10)	2.20 *q*	1.90 *q* (10)	2.17 *q*	2.23 *m*
8	3.71 *ddd* (10, 8, 5)	3.66 *ddd*	3.63 *ddd* (10, 8, 5)	4.88 *ddd*	4.94 *ddd*
9a	2.66 *dd* (14, 5)	2.78 *dd*	2.77 *dd* (13, 5)	2.72 *dd*	2.68 *dd*
9b	2.37 *dd* (14, 8)	2.43 *m*	2.07 *dd* (13, 8)	2.13 *dd*	2.23 *m*
11	2.48 *dq* (10.5, 7)	2.43 *m*	2.53 *dq* (10, 7)	2.46 *dq*	2.47 *m*
13†	1.43 *d* (7)	1.31 *d*	1.38 *d* (7)	1.23 *d*	1.30 *d*
14a	5.01 *br*	5.05 *br*‡	5.07 *br*	5.11 *br*	5.17 *br*
14b	4.98 *br*		5.01 *br*	5.09 *br*	5.09 *br*
15a	5.36 *br*	5.42 *br*	3.87‖	4.36‖	4.72‖
15b	5.36 *br*	5.29 *br*			
OAc†		2.09, 2.06	—	2.09, 2.08	2.12, 2.11
		2.03, 2.02		2.06	2.06
		2.00			
1′	4.34 *d* (7.5)	4.74 *d*	—	—	8.45 (NH)
2′	3.23 *t* (8)	5.00 *t*	—	—	—
3′	3.37 *t* (8)	5.21 *t*	—	—	—
4′	3.44 *t* (9)	5.07 *t*	—	—	—
5′	3.21 *dt* (4.5, 9)	3.76 *dt*	—	—	—
6′	3.71‖	4.19‖	—	—	—

*Run in $CDCl_3$ at 270 MHz with TMS as int. standard. Coupling constants (in parentheses) in Hz.
†Intensity of three protons.
‡Intensity of two protons.
§Three drops DMSO-d_6 added.
‖Center of AB system.

The sesquiterpene lactone constituents of *S. affinis* are, thus, not significantly different from those previously found in other *Saussurea* species and, in fact, from those generally found in Cardueae which typically elaborate a series of related germacradienolides and guaianolides.

EXPERIMENTAL

Isolation of S. affinis *constituents.* Above-ground parts of *Saussurea affinis* Spreng. (2 kg) collected in the vicinity of Arunachal Pradesh, India (voucher on deposite in herbarium of RRL, Jorhat), were extracted with $CHCl_3$ in a Soxhlet apparatus until the extract was colorless. After removal of solvent at red. pres. the residue (71 g) was dissolved in 300 ml MeOH containing 10% H_2O, allowed to stand overnight and filtered. The filtrate was washed with petrol (60–80°) (6 × 300 ml), the MeOH portion was concd at red. pres. and the residue was thoroughly extracted with $CHCl_3$ (8 × 200 ml). Evaporation of the washed and dried extract furnished 27 g of a gummy residue which was chromatographed over 500 g of Si gel (60–120 mesh, BDH). Fractions (200 ml) were collected in the following order: 1–13 (C_6H_6), 114–113 (C_6H_6–EtOAc, 9:1), 114–121 (C_6H_6–EtOAc, 4:1), 122–179 (C_6H_6–EtOAc, 2:1), 180–193 (C_6H_6–EtOAc, 1:1), 194–249 (EtOAc), 250–269 (EtOAC–MeOH, 19:1), 270–275 (EtOAc–MeOH, 9:1), 276–280 (EtOAc–MeOH, 4:1) and 281 284 (EtOAc–MeOH, 2:1).

Fractions 58–74 (0.4g) which exhibited two major spots on TLC were combined and purified by prep. TLC (C_6H_6–EtOAc, 7:1). The faster moving band yielded 54 mg of aguerin B (**1b**) as a gum; IR ν_{max} cm^{-1}: 3500, 1760, 1715, 1630, 1200, 1000 and 920; ^{1}H NMR (270 MHz, $CDCl_3$) as reported [14]; MS *m/z* 330 $[M]^+$, 244, 226, 197, 69. Acetylation (Ac_2O–pyridine overnight) provided **1e** [11] which had the expected ^{1}H NMR spectrum; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760, 1710, 1630, 1225, 1150, 1000; MS *m/z* 372 $[M]^+$, 330, 312, 244, 226, 197, 69. The slower moving band gave 45 mg of aguerin A (**1c**) [11]; IR ν_{max} cm^{-1}: 3500, 1760, 1720, 1630, 1200, 1035 and 915; ^{1}H NMR similar to that of **1b** except for substitution of the signals of the methacryl for those of the isobutyryl ester side chain; MS *m/z* 332 $[M]^+$, 314, 262, 244, 226, 197, 71. Acetylation provided **1f** which had the expected ^{1}H NMR spectrum; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760, 1725, 1630, 1220, 1150, 1000; MS *m/z*: 374 $[M]^+$, 332, 244, 226, 197, 71.

Fractions 81–94 (120 mg), which exhibited one major spot, were combined and purified by prep. TLC (C_6H_6–EtOAc, 2:1) to yield 30 mg of impure (by NMR criteria) isoamberboin (**3a**) [17, 20–22] as a gum, ^{1}H NMR (270 MHz, $CDCl_3$): δ5.06 and 4.76 (*br*, H-14ab,), 3.93 (*t*, *J* = 9 Hz, H-6), 3.75 (*dt*, *J* = 6, 9 Hz), 3.12*m* (H-1), 2.82 (*dd*, *J* = 13, 6 Hz, H-9a), 2.5 (*c*, H-11, H-5), 2.25 (*c*, H-9b, H-2a,b), 2.05 (*q*, *J* = 10 Hz, H-7), 1.44 (*d*, *J* = 7 Hz, H-13), 1.24 (*d*, *J* = 7 Hz, H-15); MS *m/z* 264 $[M]^+$, 246, 218, 203. Acetylation (Ac_2O–pyridine overnight) provided **3b** (jurmolide) [17, 23] which was pure but could not be induced to crystallize, IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1760, 1730 (broad), 1650, 1100, 950; MS *m/z*: 306 $[M]^+$, 264, 246, 218, 203; ^{1}H NMR ($CDCl_3$): δ5.15 and 4.82 (*br*, H-14a, b), 4.93 (*dt*, *J* = 6, 9 Hz, H-8), 4.01 (*t*, *J* = 9 Hz, H-6), 3.11 (*m*, H-1), 2.89 (*dd*, *J* = 13, 6Hz, H-9a), 2.54 (*t*, *J* = 9 Hz, H-5), 2.4 (*c*, H-11, H-2a), 2.24 (*c*, H-7, H-2b), 2.12 (Ac), 1.35 (*d*, *J* = 7 Hz, H-13), 1.24 (*d*, *J* = 15 Hz, H-15); ^{1}H NMR (C_6D_6) (de-

Table 2. ^{13}C NMR spectra* of compounds **2d** and **4b**

C No.	**2d**	**4b**
1	44.37 *d*†	41.46 *d*†
2	36.34 *t*†	32.79 *d*†
3	74.67 *d*†	78.24 *d*†
4	147.89	81.39
5	50.91 *d*†	55.00 *d*†
6	78.92 *d*†	76.90 *d*
7	53.93 *d*†	53.66 *d*†
8	85.10 *d*†	75.43 *d*
9	42.55 *t*	42.03 *t*
10	142.56	140.49
11	41.46 *d*	40.46 *d*
12	177.94	176.85
13	16.12 *q*	15.71 *q*
14	116.81 *t*	117.19 *t*
15	114.33 *t*	64.13 *t*
Ac–C–O	170.62, 170.42, 170.24 169.33, 169.04	170.42, 170.31, 170.04
Ac–Me	21.24, 20.70, 20.70 20.57, 20.57	21.11, 21.11, 20.92
1′	101.28 *d*	—
2′	71.94 *d*	—
3′	73.08 *d*	—
4′	68.46 *d*	—
5′	72.05 *d*	—
6′	62.19 *t*	—

*Run at 67.89 MHz in $CDCl_3$ with TMS as int. standard. Unmarked signals are singlets.

†Assignment by selective spin decoupling.

coupled to verify $J_{7,11}$): δ2.14 (*dt*, *J* = 3, 9 Hz, H-1), 2.06 (*dd*, *J* = 18, 3 *Hz*, *H*-2*a*), 1.9 (*dd*, *J* = 18, 9 Hz, H-2b), 1.81 (*m*, H-4), 1.34 (*m*, H-5), 2.90 (*t*, *J* = 9.5 Hz, H-6), 1.74 (*q*, *J* = 10 Hz, H-7), 4.48 (*dt*, *J* = 5, 10 Hz, H-8), 2.54 (*dd*, *J* = 12, 5 Hz, H-9a), 1.63 (*dd*, *J* = 12, 10 Hz, H-9b), 1.94 (*m*, H-11), 1.20 (*d*, *J* = 7 Hz, H-13), 4.63 and 4.34 (*br*, H-14a,b), 1.20 (*d*, *J* = 7 Hz, H-15), 1.62 (Ac). The value of $J_{7,11}$ (10 Hz) and the solvent shift of the H-13 signal (δ_{CDCl_3}-$\delta_{C_6D_6}$ + 0.15) indicated that the C-11 methyl group was pseudoequatorial and α [24].

Fractions 117–119 (1.5 g) gave cynaropicrin (**1a**) identified by direct comparison (TLC, IR, NMR and MS) with an authentic sample [10]. Fractions 120–151 (480 mg) yielded, after purification by prep. TLC (C_6H_6–EtOAc, 2:1) 52 mg **2a** identified by direct comparison with an authentic sample [10].

Fractions 210–224 (1.0 g) were combined. Purification by prep. TLC ($CHCl_3$–MeOH, 22:3) gave 120 mg of saussureolide (**4a**) as a gum, IR ν_{max}^{Film} cm^{-1}: 3500, 1770, 1050; MS *m/z* 298, 280, 267, 262, 249, 231. [Calcd for $C_{15}H_{22}O_6$: MW, 298.1416. Found: MW(MS), 298.1419.] ^1H NMR and ^{13}C NMR spectra are listed in Tables 1 and 2. Treatment with Ac_2O–pyridine overnight and purification by prep. TLC provided the triacetate, **4b**, whose ^1H NMR spectrum is listed in Table 1; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 1775, 1725, 1630, 1250, 1025.

Fractions 254–272 (500 mg) were combined and purified by prep. TLC ($CHCl_3$–MeOH, 4:1). This gave 80 mg **2b** as a gum; IR ν_{max}^{Film} cm^{-1}: 3500, 1770, 1050. The low resolution MS did not exhibit the molecular ion. Acetylation of 10 mg **2b** (Ac_2O–pyridine overnight) and purification by TLC furnished 10 mg of the penta-acetate, **2d**; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1775, 1750–1720 (broad band). The ^1H NMR spectrum of **2b** and the ^1H NMR and ^{13}C NMR spectra of **2d** are listed in Tables 1 and 2.

Enzymatic hydrolysis of **2a**. A mixture of 28 mg **2a** in 1.5 ml H_2O and 18 mg β-D-glucosidase was stirred for 12 hr at room temp. during which time TLC indicated disappearance of starting material. The mixture was diluted with 30 ml H_2O and extracted with EtOAc (3 × 100 ml). Evaporation of the washed and dried extract gave 15 mg **2a** identical (TLC, IR, NMR, MS) with authentic material. The aq. portion furnished glucose.

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